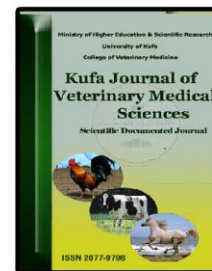




Kufa Journal for Veterinary Medical Sciences Vol.(8). No.(1) 2017

## Kufa Journal for Veterinary Medical Sciences

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### Comparison the therapeutic effects of henna, procaine penicillin and povidone-iodine solutions as uterine flushings in Iraqi Arabian mares

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#### Abstract

In the last decade, many researchers tried herbal medicine due to its beneficial therapeutic effects by less side effects, so our study is conducted on (12) Iraqi Arabian mares to compare the therapeutic effect of henna 20% with procaine penicillin and povidone-iodine 1% solutions. The results shows the superiority therapeutic effects of henna 20% than that of procaine penicillin and povidone-iodine therapies as uterine flushing .

The comparison between the types of treatments and their effects on the different types of bacteria which are included in this study, shows the superior effect on E. coli is by Henna ( $54.25 \pm 1.49$ ), the procaine penicillin has moderate effect ( $177 \pm 3.22$ ), while Povidone- iodine has weak effect ( $278.8 \pm 1.71$ ). The superior effect on Staphylococcus aureus is by procaine penicillin ( $148 \pm 25.99$ ), and Povidone- iodine ( $220 \pm 20.81$ ), While Henna has weak effect ( $278.8 \pm 1.71$ ). While the moderate effect on Streptococcus zooepidemicus is by Henna ( $70 \pm 7.93$ ) and Povidone-iodine ( $240.33 \pm 32.64$ ), while procaine penicillin has weak effect ( $180.66 \pm 2.33$ ). The fertility rates of the treatment groups are ( henna solution 20%, 91.66%, procaine penicillin solution, 66.66%, and Povidone- iodine solution, 58.33%) respectively. The pregnancy rates of the treatment groups are (henna solution 20%, 100%, procaine penicillin solution , 58.33%, and Povidone- iodine solution , 33.33%) respectively. The fertility rates of the treatment groups are ( G1, 91.66%, G2, 66.66%, and G3, 58.33%) respectively. The pregnancy rates of the treatment groups are (G1, 100%, G2, 58.33%, and G3, 33.33%) respectively All the results are significant statistically at  $P \leq 0.05$ . According to our results, we conclude that henna solution has beneficial therapeutic effect as uterine flushing for the most common causative bacteria in mares uterine. These results are very useful for veterinarians who are deal with horses health.

مقارنة التأثير الدوائي لمحاليل الحناء والبروكائين بنسلين واليود كغسول رحمي للأفراس العربية العراقية

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#### الخلاصة

في العقد الماضي، حاول العديد من الباحثين دراسة طب الأعشاب لتأثيرها الدوائي المفيد حيث تميزت بقلّة تأثيراتها الجانبية.

لذا شملت الدراسة (12) فرس عربية عراقية لمقارنة التأثير الدوائي لمحاليل الحناء 20% والبروكاين بنسلين واليود كغسول رحمية. وقد أظهرت النتائج تأثيرا دوائيا متفوقا لمحلول الحناء 20% على الأيسيرشيا المعوية (54,25 ± 1,49) وتأثيرا متوسطا لمحلول البروكاين بنسلين (3,22 ± 177) وتأثيرا ضعيفا لمحلول اليود (278,8 ± 1,71). أما المكورات العنقودية فقد كان لمحلول البروكاين بنسلين (25,99 ± 148) ومحلول اليود (220 ± 20,81) تأثيرا متفوقا عليها بينما كان لمحلول الحناء تأثيرا ضعيفا (1,71 ± 278,8). ولقد تأثرت المكورات المسببة تأثيرا متوسطا بمحلول الحناء 20% (7,93 ± 70) ومحلول اليود (240,33 ± 32,64) بينما كان لمحلول البروكاين بنسلين تأثيرا ضعيفا عليها (2,33 ± 180,66). وكان معدل الاخصاب لمجموعة العلاج بمحلول الحناء (91 و 66%) , ولمجموعة العلاج بمحلول البروكاين بنسلين (66 و 66%) , ولمجموعة العلاج بمحلول اليود (58 و 33%). وان معدل الحمل لمجموعة العلاج بمحلول الحناء (100%) , ولمجموعة العلاج بمحلول البروكاين بنسلين (58 و 33%) ولمجموعة العلاج بمحلول اليود (33 و 33%). وكان معدل الاخصاب للمجموعات العلاجية الاولى والثانية والثالثة 91 و 66% , 66 و 66% , 58 و 33% على التوالي. وكان معدل الحمل لنفس المجموعات المذكورة 100% , 58 و 33% , 33 و 33% على التوالي. ومن الجدير بالذكر , إن جميع النتائج قد أظهرت اختلافا ملحوظا بمستوى 0,05% نستخلص من نتائج البحث أفضلية محلول الحناء كغسول رحمي ضد الجراثيم الرحمية الأكثر شيوعا في الأفراس. نعتقد إن هذه النتائج ستكون مفيدة للأطباء البيطريين العاملين في مجال صحة الخيل.

## Introduction

In the last decade, many researchers tried herbal medicine due to its beneficial therapeutic effects by less side effects.

In Iraq, a previous study was concluded that henna solution had very beneficial effect on healing of tissues due to the acceleration of cells proliferation (1).

Persistent post-breeding endometritis was the third most common medical condition of adult female horses (2) and that the major reason for failure to conceive (3) It was affect approximately 15% of Thoroughbred mares (4). Due to its association with decreased fertility, it was the major concerned to breeders and veterinary practitioners (5) some bacteria such as *Escherichia coli* tenaciously adhere to epithelial surfaces, preventing their physical removal. Others such as streptococci stimulated the production of inflammatory exudates interfering with neutrophils phagocytosis. Moreover, some microorganisms secreted a biofilm that supported growth and maintenance of pathogens. Biofilms provided inherent resistance to antibiotics and both cellular and

humoral immune defenses resulting in persistent chronic infections even after prolonged antibiotic treatment (6,7). Traditional therapy (post-breeding uterine lavage, oxytocin and intrauterine antibiotics) was not always sufficient. The goals of successful therapy were corrected the defected in uterine defense breeding inflammation (7). There were different methods to treat endometritis in mare, this include: exercise and intra- uterine lavage, intra-uterine wash with antibiotics and systemically injected antibiotics (8). There are several methods described for endometritis treatment in farm animals like logul's iodine and systemic or local antibiotic (9, 10). This study was designed to compare the efficiency of different uterine flushings for endometritis treatment in Iraqi Arabian mares., neutralizing virulent bacteria and controlling post-

## Materials and methods

The study is conducted on twelve Iraqi Arabian mares in Al- Najaf province from September to December / 2016. These mares are lived free in private farms.

**Table- 1: shows the distribution of ages, are estimated according to(11), number of previous parturitions and the previous clinical history.**

NO.	Ages(months)	NO. of previous parturitions	Previous clinical history
1	69	2	-
2	72	1	Vulvitis(once)
3	36	1	-
4	83	2	Endometritis(once)
5	97	1	Endometritis(once)
6	40	-	-
7	101	3	Endometritis(twice)
8	35	-	Vaginitis(twice)
9	37	-	Endometritis(once)
10	88	2	-
11	90	1	Endometritis(once)
12	95	2	Vaginitis(twice)

**Experimental design:**

Three uterine flushing treatments ( henna solution 20% V/W with distal water {G1}, procaine penicillin G solution (300.000 IU/ ml., 15ml, dilute to 60ml. in sterile saline {G2}, and povidone- iodine solution 1% {G3}) are prepared previously and preserve at 4°C until use. Each type of uterine flushing treatments are used for 4 mares randomly according to their clinical check in our Arabian Horses Center. All the uterine flushing solutions are used at 24 hours after mating. Uterine swabs with transport media are taken from each mare after one week and transport directly in ice box to the laboratory for bacterial analysis.

**Bacterial analysis:**

In Veterinary Medicine College/ University of Al- Qadisyah laboratories, the samples swabs growth in different medias ( MacConkey's agar, Mannitol salt agar and blood agar) as selective medias for *Escherichia coli*, *Staphylococcus*

*aureus*, and *Streptococcus zooepidemicus* respectively. Bacterial colonies count are made by colony-forming unit(CFU) method to evaluate the effect of each type of our treatments.

**Statistical analysis:** The mean values $\pm$  SE are analyzed with one way ANOVA program, P values are regarded significant statistically at  $\leq 0.05$ .

**Results**

The therapeutic effects of henna 20% are very clear by the lowest number of colonies in samples NO.3,6,8,and 9. Their colonies counts are (55,53,74, and 94) respectively. The therapeutic effects of Procaine Penicillin are moderate in samples NO. 1,7,10,and12. Their colonies counts are (163,185,177,and 183) respectively. While the weak therapeutic effects of Povidone- iodine 1% are shows in samples NO.2,4,5, and 11. Their colonies counts are (284,274,238,and 298) respectively as table- 2 shows.

**Table- 2: shows the selective media, the colony count (bacteria/ml.), and the type of therapy which are used to treat the mares post mating.**

<b>Sample NO.</b>	<b>Causative bacteria</b>	<b>Selective media</b>	<b>Colony count Bacteria/ml.</b>	<b>Type of therapy</b>
1	Staphylococcus aureus	Mannitol salt agar	163	Proc.Penicillin
2	E.coli	MacConkey's agar	284	Povidone-iodine
3	E.coli	MacConkey's agar	55	Henna
4	Streptococcus zooepidemicus	Blood agar	274	Povidone-iodine
5	Streptococcus zooepidemicus	Mannitol salt agar	238	Povidone-iodine
6	E.coli	MacConkey's agar	53	Henna
7	Streptococcus zooepidemicus	Blood agar	185	Proc.Penicillin
8	Staphylococcus aureus	Mannitol salt agar	74	Henna
9	Staphylococcus aureus	Mannitol salt agar	94	Henna
10	E.coli	MacConkey's agar	177	Proc.Penicillin
11	Streptococcus zooepidemicus	Blood agar	298	Povidone-iodine
12	Staphylococcus aureus	Mannitol salt agar	183	Proc.Penicillin

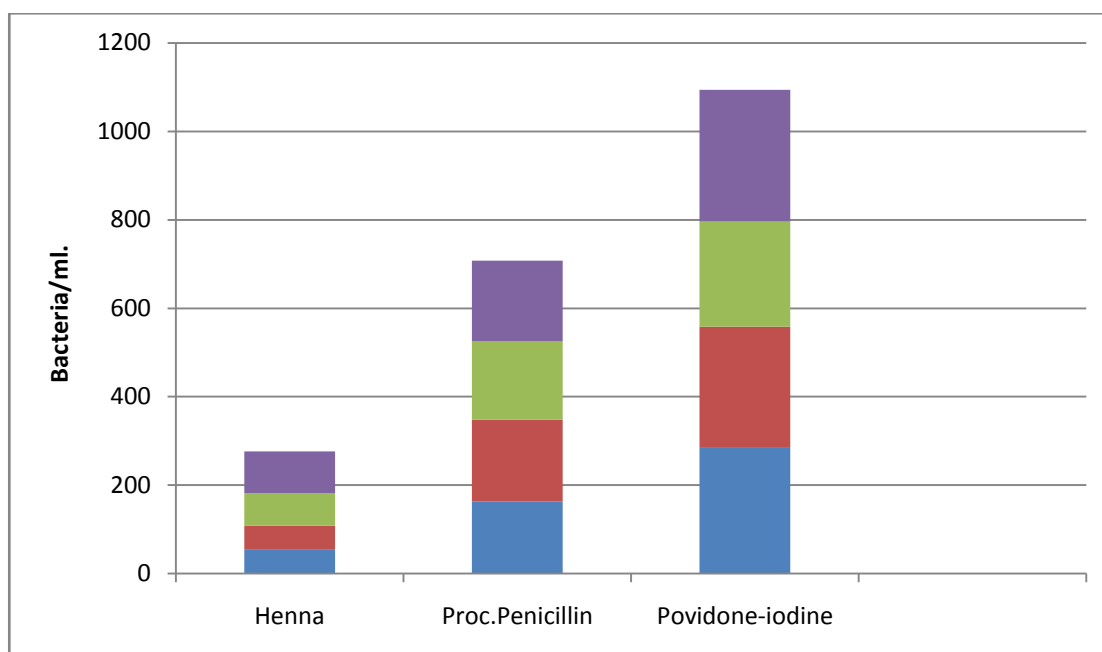


Fig.-1: Shows the comparative therapeutic effects of henna20%, Proc.Penicillin, and Povidone-iodine 1% on the bacterial growth (colony count).

The comparison between the types of treatments and their effects on the different types of bacteria which are included in this study, shows in table- 3. The superior effect on *E. coli* is by Henna ( $54.25 \pm 1.49$ ), the procaine penicillin has moderate effect ( $177 \pm 3.22$ ), while Povidone- iodine has weak effect ( $278.8 \pm 1.71$ ). The superior effect on *Staphylococcus aureus* is by procaine penicillin ( $148 \pm 25.99$ ), and Povidone- iodine ( $220 \pm 20.81$ ), While Henna has weak effect ( $278.8 \pm 1.71$ ). While the moderate effect on *Streptococcus zooepidemicus* is by Henna ( $70 \pm 7.93$ ) and Povidone- iodine ( $240.33 \pm 32.64$ ), while procaine penicillin has weak effect ( $180.66 \pm 2.33$ ). All the results are significant differences at  $P \leq 0.05$ .

Table- 3: Shows the comparison between the types of treatments and there count of bacterial colony/ ml.

Type of bacteria	Type of antibacterial drug	Count of bacterial colony / ml.
<i>E.coli</i>	Povidone – iodine	$278.8 \pm 1.71^A$
	Henna	$54.25 \pm 1.49^B$
	Proc. Penicillin	$177 \pm 3.22^C$
<i>Staphylococcus aureus</i>	Povidone – iodine	$220 \pm 20.81^C$
	Henna	$85.66 \pm 6^B$
	Proc. Penicillin	$148 \pm 25.99^C$
<i>Streptococcus zooepidemicus</i>	Povidone – iodine	$240.33 \pm 32.64^A$
	Henna	$70 \pm 7.93^B$
	Proc. Penicillin	$180.66 \pm 2.33^C$

- Different letters means significant differences at  $p < 0.05$ .
- Similar letters means non-significant differences at  $p < 0.05$ .

The fertility rates of the treatment groups are ( G1, 91.66%, G2, 66.66%, and G3, 58.33%) respectively. The pregnancy rates of the treatment groups are (G1, 100%, G2, 58.33%, and G3, 33.33%) respectively as show in table- 4.

Table- 4: The fertility rates and pregnancy rates of the treatment groups.

Group No.	Treatment	No. of mares	Fertility rates %	No. of mares	Pregnancy rates %
1	Henna solution 20%	11	91.66	11	100
2	Procaine penicillin solution	8	66.66	7	58.33
3	Povidone- iodine solution	7	58.33	4	33.33

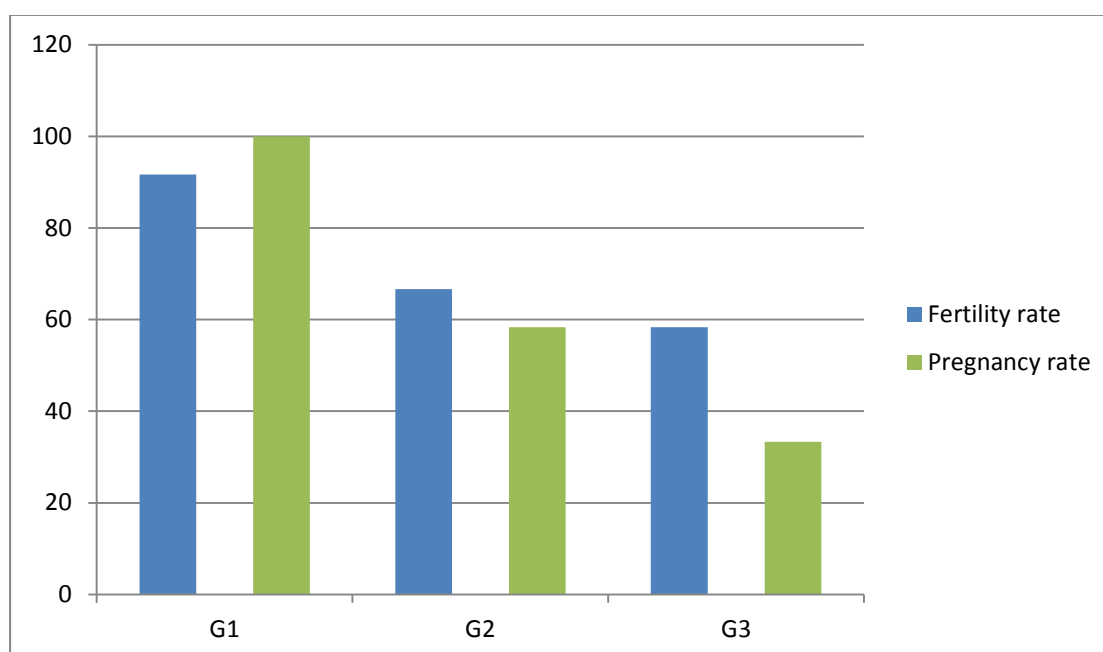


Fig.- 2: Show the fertility rates and the pregnancy rates of the treatment groups.

## Discussion

Natural breeding is regarded an important causative agent of infertility in mares. In Iraq, there is no artificial insemination policy, the breeding depend on natural insemination only, so infertility of mares is the major problem in our equine industry.

A lot of researchers try to use many medicaments for intrauterine infusions (12), (13), many of these drugs may have harmful side effects. So we aims in this study to evaluate the effect of a new natural drug (henna solution 20%) in intrauterine flushing operations post breeding and compare it with procaine penicillin and Povidone- iodine solution.

Table- 2 shows the selective medias which are used for growth of each causative agent and the colonies count/ml. of each type of therapy. The swabs are taken after one week which we believe that its enough period for the pathogens growth. Evaluation of the therapeutic effects of any antibacterial drug can be done by calculation of the colonies growth as (14), (15). Post treatments the fertilize ova can easily implant it self in the improve uterine endometrial tissue.

The results of table- 3 shows the superior effect of henna solution 20%, for E. coli, the number of bacterial colonies is  $(54.25 \pm 1.49)$ , for Streptococcus zooepidemicus  $(70 \pm 7.93)$  and for Staphylococcus aureus  $(85.66 \pm 6)$ . we believe that, henna solution has strong bactericidal effect and it can separate the adhere bacteria from the epithelial tissue which lining the uterus, Antimicrobial activity may be due to the carbohydrates and proteins in the bacterial cell wall combine with the numerous free hydroxyls which present in henna. They may get attached to enzyme sites in the bacterial cell wall rendering them inactive (16).

The moderate effects of procaine penicillin solution, also is clear in table- 3. The number of bacterial colonies of Staphylococcus aureus is  $(148 \pm 25.99)$ , of E. coli is  $(177 \pm 3.22)$ , and of Streptococcus zooepidemicus  $(180.66 \pm 2.33)$ , these results are accompanied with (17), (18).

While the weak effects of Povidone-iodine solution (lugol's 1%) is also clear in table- 3, the number of bacterial colonies are very high, Staphylococcus aureus, Streptococcus zooepidemicus, and E.coli  $(220 \pm 20.81)$ ,  $(240.33 \pm 32.64)$ ,  $(278.8 \pm 1.71)$  respectively although many veterinarians still use it, may be due to its cheap value.

The beneficial therapeutic effect of henna is clear and with high value as shows in table-4. The results show the superiority of henna solution 20%, the fertility rate 91.66% (11 mares) while the pregnancy rate is 100% (11 of 11 mares). We believe that henna solution 20% has powerful effect as bactericidal it can clean the uterus and prevent formation of biofilms of the micro-organisms. According to (19) mares as young as 8 years but less than 12 years, may be have elongated, closed, fibrous cervix during estrus. Their uterus is normal in size but it is common to accumulate some echolucent fluid in the uterine lumen, the development of uterine edema which compromised the fertility in case of natural breeding.

According to our results, we conclude that henna solution has beneficial therapeutic effect as uterine flushing for the most common causative bacteria in mares uterine. These results are very useful for veterinarians who are deal with horses health.

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